Harmful Algae xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake

Daniel L. Roelke [a,b,*], George M. Gable [a], Theodore W. Valenti [c], James P. Grover [d], Bryan W. Brooks [c], James L. Pinckney [e]

[a] Department of Wildlife and Fisheries Sciences, Texas A&M University, 2258 TAMUS, College Station, TX 77843-2258, USA
[b] Department of Oceanography, Texas A&M University, 2258 TAMUS, College Station, TX 77843-2258, USA
[c] Department of Environmental Science, Baylor University, USA
[d] Department of Biology, University of Texas at Arlington, USA
[e] Department of Biological Sciences, University of South Carolina, USA

ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form 8 December 2009
Accepted 8 December 2009

Keywords:
Environmental stress
Inorganic nutrients
Irradiance
Salinity
Temperature

ABSTRACT

Prymnesium parvum blooms have increased in frequency and magnitude in the south-central USA in recent years, resulting in large fish kills and economic losses. Here, we document seasonal and system-wide plankton dynamics of Lake Granbury, TX, over a period spanning the formation and termination of a large, highly toxic, *P. parvum* bloom that occurred from January through March 2007. High-resolution spatial mapping showed that this bloom was system-wide and patchy during its peak densities. Consistent with laboratory studies, the highest in-lake toxicity to fish occurred during peak bloom density and under the most stressful in-lake conditions (based on salinity, temperature, light and inorganic nutrients). As with other *P. parvum* blooms, this bloom at its peak density was near monospecific, with *P. parvum* accounting for ~92% of the phytoplankton biomass, and diatoms, cyanobacteria and green algae comprising most of the remaining biomass. A large inflow event in April obliterated this bloom, dramatically reducing population densities by 89% and completely removing toxicity to fish. Interestingly, the bloom had already started to decline somewhat before this hydraulic flushing event affected the lake. During this decline, in-lake conditions were not likely stressful to *P. parvum*, and predation did not appear to be a factor. The role of pathogens of *P. parvum* was not assessed during this study, however. Our findings show a strong link between hydrology and bloom termination, which raises concerns given that in-stream flows are predicted to decline as human population increases in this region. In addition, flow reduction may be exacerbated by climate change. Increased understanding of factors that influence *P. parvum* blooms is paramount given the possible need to offset the effect of diminished hydraulic flushing.

1. Introduction

The haptophyte alga *Prymnesium parvum* Carter occurs worldwide, is tolerant of large variations in temperature and salinity, and sometimes forms fish-killing blooms (Edvardsen and Paasche, 1998; Lundholm and Moestrup, 2006; Baker et al., 2007, 2009). Since 2001, the incidence of *P. parvum* blooms in the south-central USA has increased dramatically. In this region, fish-killing blooms are now observed in many lakes found along multiple river basins. During blooms, surface waters take on a golden color with *P. parvum* cell densities typically exceeding 10×10^6 cells L^{-1} (TPWD, 2003; Roelke et al., 2007; Schwierzke et al., in press). Many fish species ranging from herbivores to piscivores are killed during these blooms, where total mortalities number in the tens of millions (TPWD, 2003; Roelke et al., 2007). Environmental conditions conducive to blooms and the factors that lead to the formation and termination of harmful algal blooms (HABs) in general are complex (Paerl, 1988; Roelke and Buyukates, 2001). Currently, the factors affecting the incidence of *P. parvum* blooms in the south-central USA are only partly understood.

Conditions that favor *P. parvum* blooms in the south-central USA might include eutrophication and salinization. Blooms in Europe, the Middle East, and Asia have all occurred in eutrophic and brackish systems (Krasnotshchek and Abramowitsch, 1971; Holdway et al., 1978; Rijn van and Shilo, 1989; Kaartvedt et al., 1991; Guo et al., 1996; Amsinck et al., 2005; Granéli et al., 2008). *P.*

* Corresponding author at: Department of Wildlife and Fisheries Sciences, Texas A&M University, 2258 TAMUS, College Station, TX 77843-2258, USA.
Tel.: +1 979 845 0169; fax: +1 979 845 4096.
E-mail address: droelke@tamu.edu (D.L. Roelke).

1568-9883/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2009.12.003

parvum blooms in the south-central USA are partly consistent with these observations because they appear mostly in lakes that attain salinities of 2–4 practical salinity units (psu) during low precipitation years (TPWD, 2003), and these lakes may have experienced increased nutrient loading because of aging septic systems, point source discharges and expanded shoreline development. The role of nutrients is complex, however. The toxicity to fish from chemicals produced by *P. parvum* was greater when cells were nutrient-limited (Uronen et al., 2005; Roelke et al., 2007; Errera et al., 2008). These chemicals are also believed to act as allelochemicals important to bloom initiation. In addition, *P. parvum* is sensitive to pulses of nutrients where high doses inhibited bloom formation and reduced toxicity (Barkoh et al., 2003; Grover et al., 2007; Kurten et al., 2007; Gordon and Colorni, 2008). Therefore, the temporal variation in nutrient availability (timing and duration of nutrient limitation) is likely more important than a system's trophic state (total nutrients in the system).

Factors initiating and terminating *P. parvum* blooms in the south-central USA have not been determined. They may include the production of chemicals toxic to grazers (Granéli and Johansson, 2003; Tillmann, 2003; Barreiro et al., 2005; Calliari and Tiselius, 2005; Roelke et al., 2007; Sopanen et al., 2006, 2008; Michaloudi et al., 2009; Brooks et al., in press), use of alternative energy and nutrient sources through mixotrophic and saprophytic nourishment (Nygaard and Tobiesen, 1993; Skovgaard and Hansen, 2003; Burkholder et al., 2008; Lindehoff et al., 2009), suppression of competitors through allelopathy (Fistarol et al., 2003, 2005; Granéli and Johansson, 2003; Roelke et al., 2007; Errera et al., 2008; Michaloudi et al., 2009), and resistance to the allelopathic effects of cyanobacteria (Suikkanen et al., 2004; Tillmann et al., 2007; but see Grover et al., in press; Roelke et al., in press). These factors are not mutually exclusive. Additional factors that seem to influence the growth of HABs include production of beneficial or deleterious chemicals by various bacterial taxa (Kodama et al., 2006; Salomon and Imai, 2006) and the pathogenic effects of viruses (Salomon and Imai, 2006; Schwierzke et al., in press).

In the south-central USA, *P. parvum* blooms occur during the January through March months (winter through early spring). Interestingly, temperature conditions during this time of the year are not optimal for *P. parvum* growth (based on observations of the Texas strain of *P. parvum*), and are in fact very near the edge of its niche (Baker et al., 2007, 2009). This underscores the importance of other factors that might give *P. parvum* a selective advantage over its competitors, such as grazer inhibition, allelopathy and mixotrophy, thereby allowing blooms to initiate. It also raises the question of what factors terminate blooms in lakes of the south-central USA, which occur at a time when temperature becomes more favorable for growth. In this manuscript we provide the first seasonal and system-wide characterization of *P. parvum* bloom formation and termination in a subtropical lake, Lake Granbury, TX, located in the south-central USA.

2. Methods

Lake Granbury is a reservoir on the Brazos River, TX, USA, constructed in 1969. The lake has a capacity of 167×10^6 m^3, a surface area of 34 km^2, and an average depth of ~5 m. The shoreline follows the meandering river channel with an elongated, sinuous basin oriented northwest to southeast that is ~45 km long and has an average width of 0.6 km. Daily discharges from the Brazos River into the lake were measured at a location upstream from the lake (Dennis, TX, USGS Station Number 08090800).

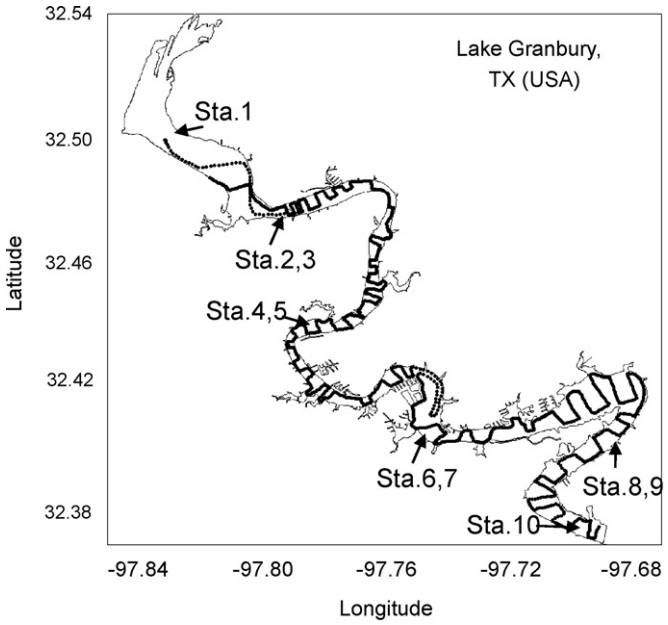

Fig. 1. Lake Granbury located in the south-central USA. This research coupled fixed station sampling with high-resolution spatial mapping (dark line indicates path of on-board data collection) that enabled detailed analysis of the plankton environment and system-wide characterizations of bloom dynamics.

Monitoring in Lake Granbury encompassed monthly sampling at 10 fixed stations (Fig. 1) and spanned August 2006 through August 2007. For purposes of this research, only data collected at six stations were used in the analysis (stations 1, 3, 5, 6, 8, and 10). These stations were located over the historic riverbed. The other four stations represented shallow water environments adjacent to some of the deep-water locations and are a part of a separate study. At each station multiple parameters were measured from well-mixed surface waters (i.e., samples were taken at 0.5 m, and vertical profiling with a multi-probe was as deep as 10 m), including characterizations of the phytoplankton (chlorophyll *a*, *P. parvum* population density, and the density of taxonomically aggregated phytoplankton groups such as cyanobacteria, diatoms and chrysophytes) and water quality (salinity, temperature, light, pH, inorganic nutrients, and ambient water toxicity). In addition, system-wide, high-resolution spatial maps of chlorophyll *a* were generated during each sampling date. Throughout the study period, our vertical profiling indicated the lake was well mixed, as all water quality profiles showed no gradients with depth.

Estimates of total phytoplankton biomass (approximated using chlorophyll *a*) and biomass of taxonomically aggregated phytoplankton groups (approximated as a fraction of the total chlorophyll *a*) were determined from photopigment biomarker concentrations (Pinckney et al., 1998) and by the use of CHEMTAX (Mackey et al., 1997; Wright et al., 1996). For the CHEMTAX model initiation, aggregated taxonomic groups were selected based on their historical prevalence in L. Granbury. For greater details of the HPLC and CHEMTAX methods followed, see Roelke et al. (2007).

For the estimation of *P. parvum* population density, a 100 mL phytoplankton sample was collected from each station and preserved using glutaraldehyde, 5% (v/v). Enumeration of *P. parvum* was performed using a settling technique (Utermöhl, 1958) and inverted, phase contrast light microscopy (400×, Leica Microsystems). A subsample of 1 mL was settled for 24 h. Randomly selected fields-of-view were then counted until >200 *P. parvum* cells were counted (5–40 fields-of-view).

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

Samples for inorganic nutrients (nitrogen and phosphorus) were filtered through pre-combusted GF/F filters, and the filtrates were frozen until analysis. Inorganic nutrient concentrations were determined using autoanalyzer methodology (Armstrong and Sterns, 1967; Harwood and Kuhn, 1970). For this study, nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4) were summed as dissolved inorganic nitrogen (DIN), and phosphorus was soluble reactive phosphorus (SRP).

Ambient water toxicity was estimated from acute toxicity to fish. To achieve these observations, standardized 24-h static toxicity assays with the juvenile fathead minnow (*Pimephales promelas*) model were employed. Toxicity assays followed standardized methods for determining the aquatic toxicity of ambient surface waters (US EPA, 2002). Samples were collected and transported to the laboratory where toxicity tests were initiated within 24 h. Ambient samples were diluted using a 0.5 dilution series with reconstituted hard water, which was performed generally following US EPA recommendations (US EPA, 2002). For greater details of the methods followed for these fish toxicity assays with *P. parvum*, refer to Roelke et al. (2007) and Brooks et al. (in press). Toxicity was summarized as LC_{50} calculated as the percent dilution of the ambient sample causing 50% mortality.

Salinity, temperature and pH in Lake Granbury were determined with a water quality multi-probe (Quanta, Hydrolab) and light penetration was determined with a Secchi disk. Estimation of average irradiance based on Secchi depth (z_{Secchi}) is discussed further below.

To complement our spatiotemporal characterizations of the *P. parvum* bloom, and phytoplankton as a whole (based on our fixed station data), we measured spatial patterns of chlorophyll *a* during each sampling trip with Dataflow, a high-speed, flow-through measurement apparatus developed for mapping physicochemical parameters in shallow aquatic systems (Madden and Day, 1992). We used this integrated instrument system to concurrently measure multiple water quality parameters that included chlorophyll *a* (*in vivo* fluorescence) from a boat following closely spaced transects (see Fig. 1). Measurements were taken at 2-s intervals from ~20 cm below the surface. An integrated GPS was used to simultaneously plot sample locations. GPS and Dataflow information were then used to create detailed contour maps (Surfer v8.0).

Non-metric multidimensional scaling (NMS) was used to explore multivariate relationships within the fixed station data (PC-ORD v.5.1; McCune and Mefford, 1999). Matrices were based on normalized physical, chemical and biological variables. We employed the Sorensen (Bray-Curtis) dissimilarity metric to determine the dimensional distances among each sampling time at each fixed station. A final solution of two dimensions was achieved based on the lowest stress obtained using a Monte-Carlo test after 250 iterations (repeated 10 times) in a cascade procedure and using a stability criterion of 0.0001 (McCune and Grace, 2002). Final stress was calculated as 12.88. Joint vectors were then used to identify significant variables that mediate the temporal and spatial changes. Vectors were drawn as the hypotenuse of regression determination coefficients (r^2) between variables (see McCune and Grace, 2002).

Based on our observations, hydraulic flushing played an important role as a mechanism influencing *P. parvum* population dynamics. To better evaluate its relative impact, we estimated in-lake specific growth rates for *P. parvum* using a mathematical model. The model (Eq. (1)) was based on the laboratory experiments using a strain of *P. parvum* isolated from Texas waters where salinity, temperature and light were varied (Baker et al., 2007, 2009), and was used previously to investigate *P. parvum* population dynamics (Grover et al., in press). The model was as follows:

$$\mu = -3.531 + 0.02534(S - 1.833) - 0.06311(S - 1.833)^2 + 7.468e^{0.7((T-20)/20)} - 3.414e^{1.4((T-20)/20)} + 0.1697(S - 1.833)e^{0.7((T-20)/20)} + 0.000611(E - 222) - 0.00000573(E - 222)^2 \quad (1)$$

where μ is the specific growth rate (d^{-1}), S is the salinity (psu), T is the temperature (°C) and E is the irradiance ($\mu mol\ m^{-2}\ s^{-1}$). These equations were formulated under experimental conditions where inorganic nutrients did not limit *P. parvum* specific growth rate.

For purposes of this research, we used the average underwater irradiance (I_{avg}) as a surrogate for E. This was approximated using the surface irradiance, a relationship between the light extinction coefficient and Secchi depth, and the average depth of Lake Granbury. Surface irradiance (at zero depth, I_0) was estimated using mathematical models that accounted for time of year and latitude, and assumed cloudless conditions and a fixed water reflectance (Kirk, 1994; Wetzel, 2001). The light extinction coefficient (k) was estimated as a function of Secchi depth ($1.7/z_{Secchi}$, Wetzel, 2001). The average underwater irradiance was then approximated using:

$$I_a = \frac{I_0(1 - e^{-kz})}{kz} \quad (2)$$

where z (m) is the average depth of Lake Granbury. Average irradiance ranged from 187 to 284 μmol photons $m^{-2}\ s^{-1}$, with a mean value of 218 μmol photons $m^{-2}\ s^{-1}$.

The calculated specific growth rate was used along with flushing losses to estimate the change in population density attributable to flushing during the period when the bloom was terminated using a simplified differential equation employed in previous studies (Roelke et al., 2003; Roelke and Eldridge, 2008, in press):

$$\frac{d\phi}{dt} = (\mu - d)\phi \quad (3)$$

where ϕ is the *P. parvum* population density with an initial condition equal to the March sampling date (just prior to the flushing event), μ is the daily specific growth rate interpolated between the March and April samplings (based on Eqs. (1) and (2)), and d is the daily flushing rate for the period between March and April samplings calculated by dividing the daily inflow (USGS records) by the volume of naturally occurring well-mixed segments of the lake. Based on the Dataflow maps for chlorophyll *a*, the length over which lake waters were well mixed was determined to range between 1 and 6 km for the March and April samplings, respectively. By employing average lake depth and width dimensions, the volume of well-mixed segments was estimated.

3. Results

For the period of study, inflows into Lake Granbury were episodic, as is common in lakes of the south-central USA. From September 2006 through March 2007 inflows were barely discernable (Fig. 2). In April 2007 the first large inflow event occurred with peak flows attaining $80 \times 10^6\ m^3\ d^{-1}$, corresponding to single-day hydraulic flushing peaks of $16\ d^{-1}$ (if well-mixed patches are on the scale of 1 km) and $2.7\ d^{-1}$ (if well-mixed patches are on the scale of 6 km). Episodic inflows of varying magnitude and duration persisted through June, where the largest inflow event reached $\sim 120 \times 10^6\ m^3\ d^{-1}$.

According to our CHEMTAX pigment model, phytoplankton biomass peaked in March just prior to the first large inflow event of

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

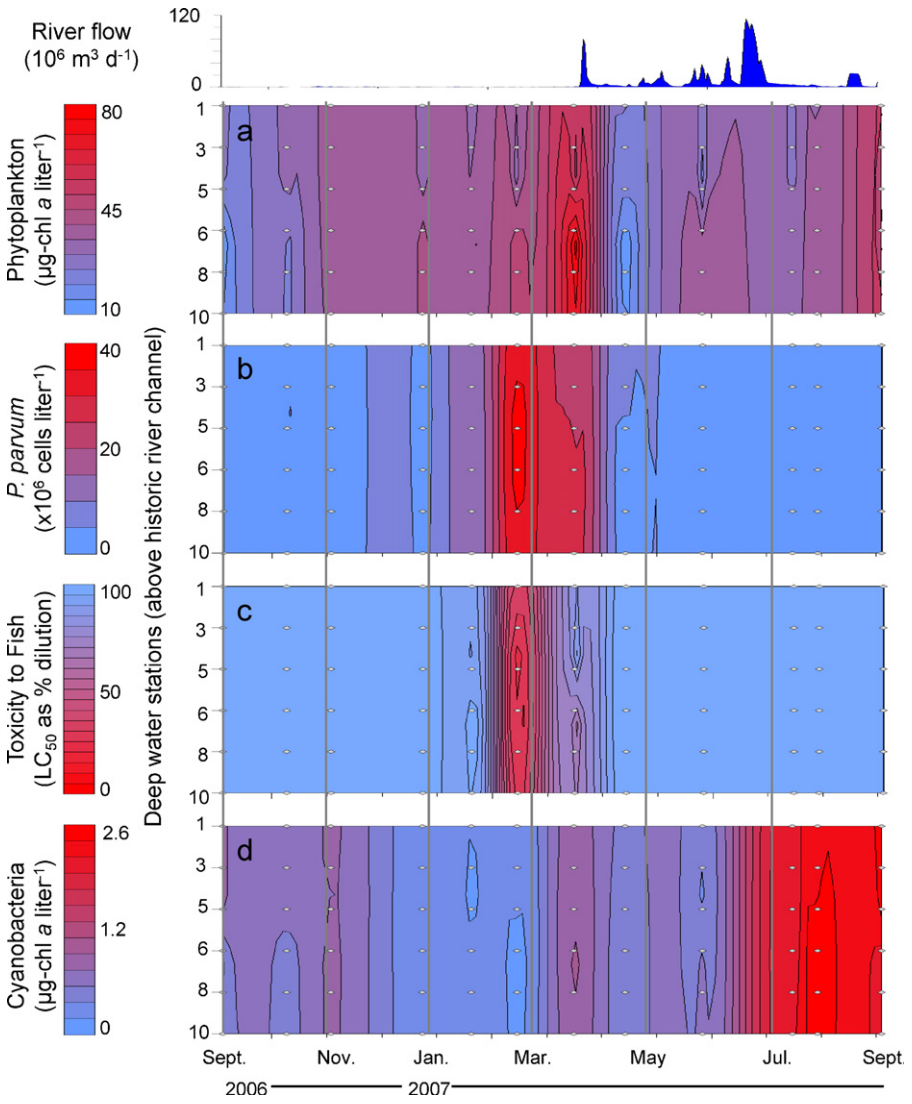

Fig. 2. Characterizations of the phytoplankton and ambient toxicity for a 1-year period that spanned the formation and termination of a *P. parvum* bloom. The bloom developed during the fall months and peaked in winter, but following a high inflow event in April the bloom was greatly diminished, as evidenced by trends in chlorophyll *a* (a) and *P. parvum* population densities (b). Toxicity (c) was maximal during the peak of *P. parvum* population density, but ceased after this inflow event. Cyanobacteria (d), potential competitors through allelopathy, were prevalent only during the summer months.

2007, with highest biomass occurring towards the lower end of the lake (Fig. 2a). Phytoplankton composition varied somewhat during the 3 months leading up to the biomass peak (January through March 2007). Prymnesiophytes were prevalent from January to February, comprising ~92% of the total phytoplankton biomass. During this same period other taxonomic groups changed little and were ~6% diatoms, ~1% green algae and ~0.5% cyanobacteria. For the period between February and March the prevalence of prymnesiophytes declined to 87% of the total phytoplankton biomass. The prevalence of diatoms and cyanobacteria increased to 10% and 2% during this period, respectively.

Our direct cell counts of *P. parvum* were in agreement with our CHEMTAX predictions and indicated that the *P. parvum* bloom reached its highest population densities of $\sim 40 \times 10^6$ cells L^{-1} in February with highest population densities occurring in the mid-reaches of the lake (91% of the phytoplankton biovolume). Cell densities $>10 \times 10^6$ cells L^{-1} are considered bloom proportions based on historical observations in lakes of the south-central USA (TPWD, 2003; Roelke et al., 2007; Schwierzke et al., in press). Average *P. parvum* densities for the lake declined ~27% by March (Fig. 2b), a larger proportional decrease than predicted by the CHEMTAX model. Measurements of ambient toxicity to fish were consistent with observed population densities, with LC_{50} values as low as 4% observed in February in the mid-reaches of the lake, with toxicity to fish decreasing (LC_{50} increasing) by March (Fig. 2c). *P. parvum* population densities for the lake were obliterated after the first large inflow event to the lake, decreasing by 89% from the March to April sampling. In addition, waters were no longer toxic to fish.

Cyanobacteria were not abundant in Lake Granbury during the time of bloom development or termination. Cyanobacterial biomass was maximal during the months of July through September 2007 (Fig. 2d) and in the lower reaches of the lake, accounting for ~7–8% of the total phytoplankton biomass according to CHEMTAX pigment estimates.

Dataflow mapping revealed that the bloom was patchy throughout Lake Granbury with characteristic patches of ~1 km (Fig. 3a, representative map during the bloom). The location of elevated chlorophyll *a* patches did not appear related to morphometric attributes such as shoreline development or depth. While we have microscopic and photopigment verification of the *P. parvum* bloom only from the fixed stations, visual observations

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

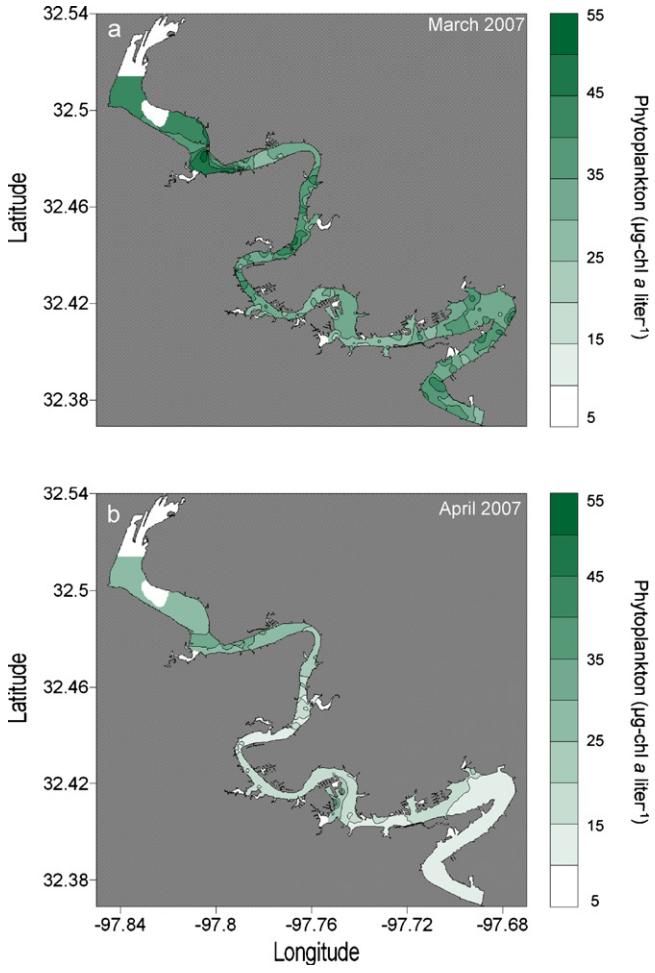

Fig. 3. System-wide characterizations of phytoplankton biomass using high-resolution spatial mapping. During the winter months, biomass patterns suggest a system-wide, but patchy bloom of *P. parvum* (a). After the flushing event, phytoplankton densities were much lower and the distribution remained patchy (b).

indicated 'golden' colored water, foam lines and dead fish throughout the lake during January to March 2007. This suggests that the *in vivo* fluorescence signal attributed to chlorophyll *a* from the Dataflow unit indicates the *P. parvum* bloom distribution. After the first inflow event in April 2007, phytoplankton biomass was greatly diminished and patchiness decreased, where characteristic patches were ~6 km (Fig. 3b).

As with many of the lakes in the south-central USA, Lake Granbury can sometimes be brackish. Prior to April 2007, when inflows were not significant, salinity was ~1.5 psu throughout Lake Granbury (Fig. 4a). The lake remained at salinities >1 psu until after the first large inflow event in April 2007, at which time salinity dropped to ~0.5 psu. Temperature changes in the lake were seasonal, with minima of ~6 °C in February 2007 and maxima of ~32 °C in August 2007 (Fig. 4b). While Secchi depth varied spatially and temporally, a prominent feature was relatively deep Secchi depths (~1 m) coinciding with the *P. parvum* bloom, while a rapid decrease in light penetration (to ~0.5 m) immediately followed the first large inflow event in April 2007 (Fig. 4c). Similarly, pH was greatest (~8.8) during the period of bloom and decreased (to ~7.8) immediately following the first large inflow event in April (Fig. 4d).

Dissolved inorganic nutrients also showed a strong relationship with inflow, and immediately following the first large inflow event in April 2007 both SRP and DIN reached their maxima of ~0.55 μM-P and ~24 μM-N (Fig. 5). Highest nutrient concentrations were measured in the lower reaches of the lake at this time. During January through March 2007, when *P. parvum* population densities were greatest and then started to decline, the DIN:SRP was ~30 with SRP concentrations ~0.05 μM-P and DIN ~1.35 μM-N. Except for December 2006, nutrient concentrations during the bloom were similar to the months prior to the bloom. In December, SRP concentrations were at their lowest, ~0.03 μM-P, while DIN was ~3.55 μM-N (DIN:SRP ~122).

The multivariate NMS analyses also revealed strong temporal patterns in Lake Granbury (Fig. 6). A prominent feature of the two-dimensional solution (71% and 22% of the variability in the data shown on axes 1 and 2, respectively) was the *P. parvum* bloom occurring in the winter months (January through March 2007). The maxima in copepod adults and rotifers that occurred during these months (data not shown), but not coincident with the *P. parvum* maximum, was also clearly shown. Other prominent features of the analysis showed the higher nutrient concentrations following the inflow events to Lake Granbury during the spring and early summer months (April through July 2007), a period when *P. parvum* cell densities were greatly reduced. Finally, the cladocera maximum in the spring (data not shown) and the cyanobacteria and ciliated protozoa maxima (data not shown) in the summer months were prominent features of the analysis.

The mathematical model used to predict the specific growth rate of *P. parvum* (Eq. (1)) indicated that growth rate of *P. parvum* was lowest at the time when cell densities were highest, i.e., January and February 2007 (Fig. 7a). Interestingly, specific growth rate predictions increased for March 2007, at the time when *P. parvum* population densities were beginning to decline. The temperature terms in these models strongly affected predictions. As temperature increased during January to March, growth rate increased. In the late summer (August and September 2007), temperatures above the modeled optimum for *P. parvum* led to the predictions of decreased growth rate.

The size of well-mixed surface water patches varied. During March, after an extended period of low inflows, patches were on the scale of ~1 km. During April, after a large inflow event, the lake was better mixed and patches were on the scale of ~6 km. Patch size influences the estimate of hydraulic flushing. Because our frequency of sampling did not match the rate of hydraulic change, we evaluated hydraulic flushing for this period bounded by lower (6 km) and upper (1 km) bounds for flushing estimates. This analysis showed that hydraulic flushing was near non-existent prior to the first large inflow event in April, and that inflow pulses started in April and continued sporadically through the early summer months producing flushing losses higher than specific growth rates for *P. parvum* (Fig. 7b).

Focusing on the period between March and April, when the first large inflow event occurred, average *P. parvum* population density in the lake decreased ~89%, as mentioned above. Modeled population reductions for this month-long period (based on Eq. (3)) were 100% and 68% for the scenarios where well-mixed lake segments were 1 and 6 km, respectively (see Fig. 3). The model, however, predicted that this decrease to the *P. parvum* bloom occurred over a period of only 4 days (Fig. 8).

4. Discussion

This study is the first to document the dynamics of *P. parvum* across an entire lake and over an annual cycle, encompassing bloom formation, toxicity to fish, and bloom termination. Some of the patterns found here confirm the current understanding of population dynamics and toxicity of this HAB species, while others raise questions.

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

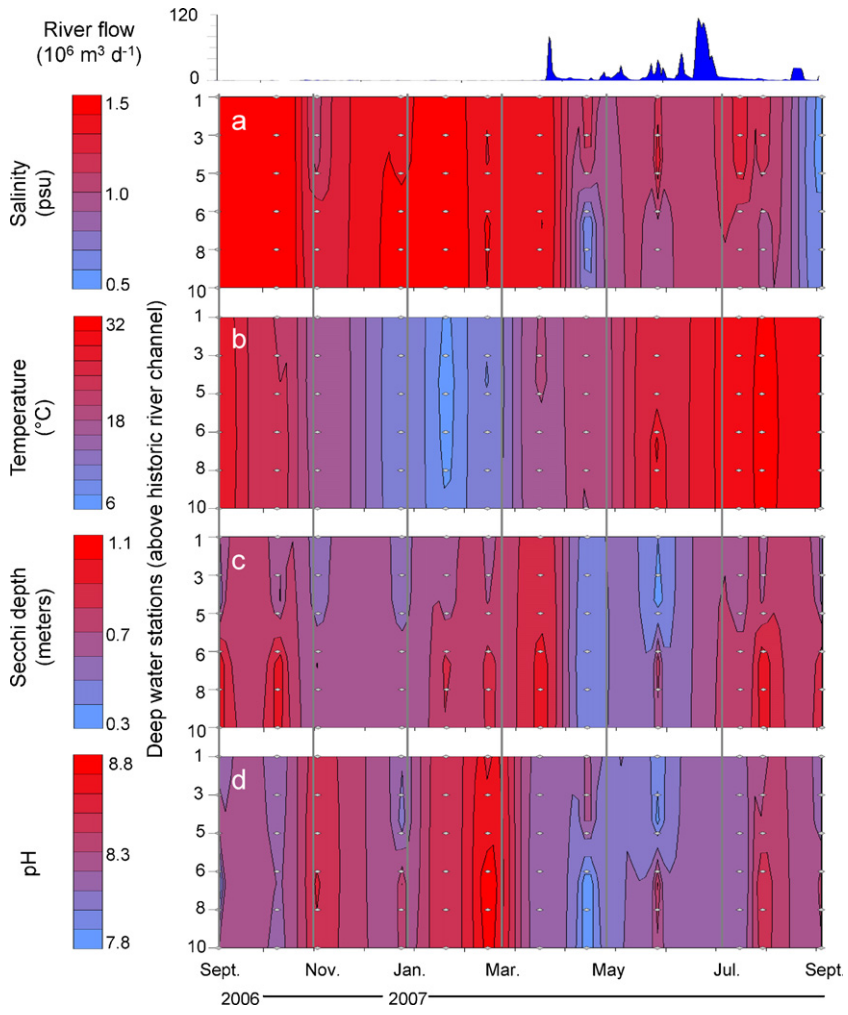

Fig. 4. Characterizations of the physicochemical environment for a 1-year period that spanned the formation and termination of a *P. parvum* bloom. Salinity (a) was highest in the earlier part of the study period, but decreased following the April inflow event. Temperature (b) followed a typical seasonal cycle for north-hemisphere, sub-tropical climates. Irradiance, as approximated with Secchi depth (c), decreased with the spring inflow events. pH (d) was highest during the peak bloom period, and decreased following the April inflow event.

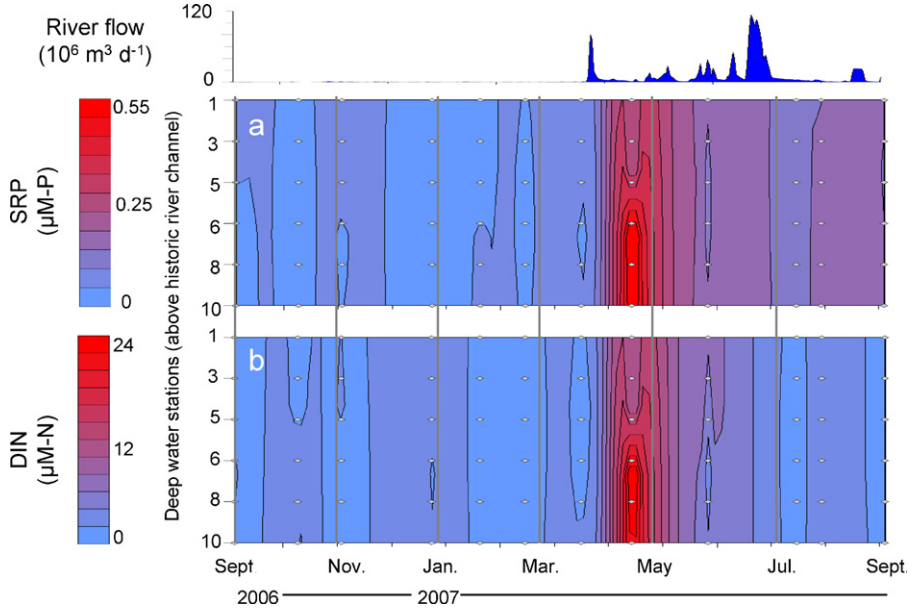

Fig. 5. Characterizations of inorganic nutrients for a 1-year period that spanned the formation and termination of a *P. parvum* bloom. Soluble reactive phosphorus (a) and dissolved inorganic nitrogen (b) were low in the lake prior to the April inflow event, but were still at concentrations high enough to support *P. parvum* growth.

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

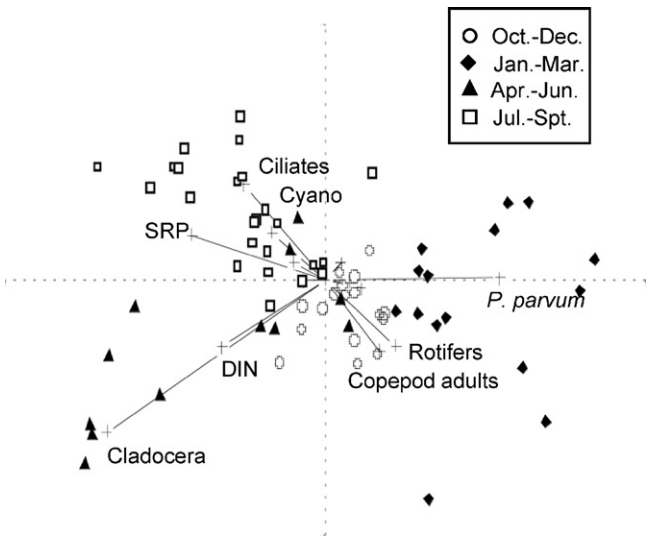

Fig. 6. Non-metric multidimensional analysis (71% and 22% of the variability shown on axes 1 and 2, respectively) reinforced field observations by showing the bloom of *P. parvum* to be associated with the winter months along with adult copepods and rotifers, the nutrient loading to be associated with the spring and early-summer inflow events, cladocera abundance to be associated with the spring months, and ciliates and cyanobacteria to be associated with the summer months. Each point in this graph represents a single sampling in space and time. That is, no averaging of data is shown.

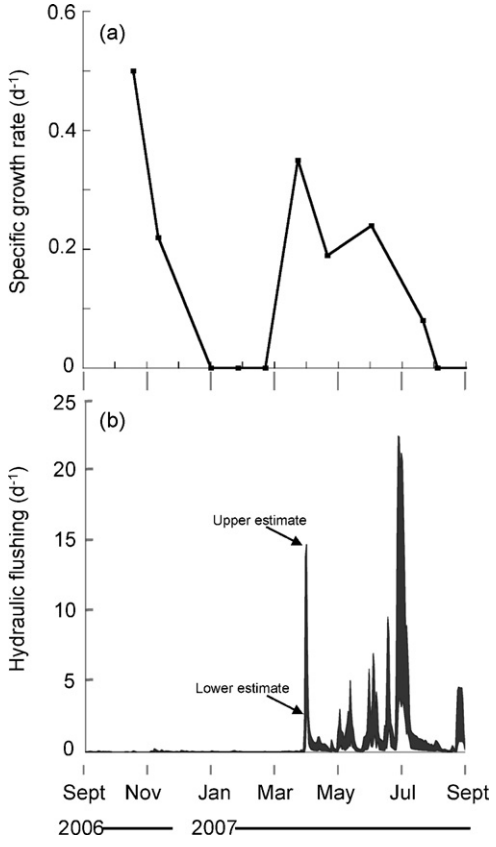

Fig. 7. Mathematical model (Eq. (1)) that predicts *P. parvum* growth rate as a function of salinity, temperature and light (a) revealed that periods of stress coincided with January and February (the period of peak bloom intensity) and late summer. Estimates of hydraulic flushing (b) showed that prior to the first large inflow event in April hydraulic flushing was negligible. When inflows commenced, however, they resulted in flushing losses greater than the specific growth rate of *P. parvum*. Lower and upper estimates of flushing were calculated based on well-mixed patches of 6 and 1 km, respectively.

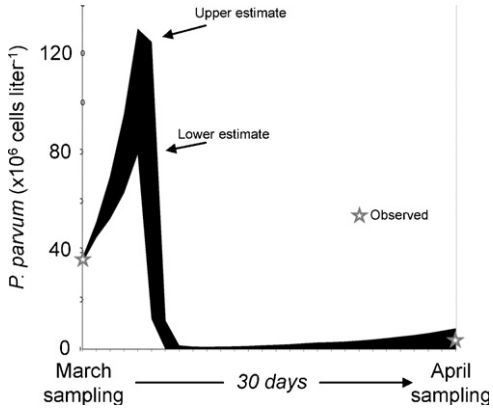

Fig. 8. Numerical model (Eq. (3)) simulation that reproduced our monthly observation of the *P. parvum* bloom decline (open stars) showing that the actual bloom termination occurred over an estimated period of 4 days. Lower and upper estimates of population density were from simulations that employed well-mixed patches of 6 and 1 km, respectively.

Production of toxic chemicals by *P. parvum* increases when environmental conditions are stressful (Nygaard and Tobiesen, 1993; Barreiro et al., 2005; Uronen et al., 2005; Roelke et al., 2007; Errera et al., 2008). Like other HAB species (Granéli and Hansen, 2006; Turner, 2006; Prakash et al., 2009), production of toxic chemicals might enable *P. parvum* to initiate blooms because it imparts a competitive advantage over other phytoplankton or inhibits grazers.

During the months prior to the *P. parvum* bloom in Lake Granbury (September through December 2006), it is unclear which environmental conditions might have been stressful. Salinity, temperature and light were favorable for *P. parvum* growth. Nutrient concentrations during those months did not seem strongly limiting. SRP reached its lowest concentrations in December 2006, when it was ~0.03 μM-P (DIN:SRP ~121). While this concentration might have limited growth of some phytoplankton species (Grover, 1989; Grover et al., 1999; Reynolds, 2006), it was not likely limiting to *P. parvum*, which has shown positive growth at SRP concentrations lower than this (Baker et al., 2009). Lack of stress and generally favorable conditions suggest that the *P. parvum* bloom could have initiated from *in situ* growth in Lake Granbury, and that allelopathic effects were not important at this early stage. Another possibility is that conditions favorable to bloom initiation occurred in a lake up-river of Lake Granbury, i.e., Lake Possum Kingdom (Roelke et al., 2007), and that the bloom developed due to mass effects (*sensu* Leibold and Miller, 2004) resulting from large migrations of *P. parvum* cells into Lake Granbury.

In contrast to these early stages, environmental conditions were stressful to *P. parvum* during the periods of bloom development and peak population densities (January and February 2007). While nutrient concentrations did not appear to be strongly limiting, predicted growth rates based on temperature, salinity and light were near zero, primarily due to the low winter temperatures. Consistent with the notion that *P. parvum* cells are more toxic when stressed by suboptimal growth (Baker et al., 2007), the highest observed fish toxicities were during these winter months.

At this time, the *P. parvum* bloom was extensive throughout the entire system, a phenomenon observed for many other HAB species (e.g., Roelfsema et al., 2006; Chang et al., 2008; Oh et al., 2009). The bloom was also patchy, as some areas of the lake experienced elevated phytoplankton biomass while others experienced the bloom at lower densities. Blooms of *P. parvum* are typically characterized by near-complete dominance of this species, which has been observed during blooms in other lakes

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

of the Brazos River (Roelke et al., 2007; Schwierzke et al., in press) and elsewhere (Sunda et al., 2006; Sopanen et al., 2008; Michaloudi et al., 2009). This *P. parvum* bloom in Lake Granbury was also near-monospecific (~92% decreasing to 87% before the first flushing event), with diatoms, green algae and cyanobacteria comprising much of the remaining biomass.

The first large inflow event, which occurred in April 2007, obliterated the *P. parvum* bloom. Single day estimates of hydraulic flushing for Lake Granbury at this time ranged between 2.7 and 16 d^{-1}. These estimates of flushing loss were greater than the predicted growth rate of *P. parvum* based on the conditions of salinity, temperature and light. Our modeled estimate of net population growth rate not only predicted population declines that agree quantitatively with the monthly observed declines, but also indicated that the actual bloom termination occurred over a much shorter period, i.e., 4 days. Hydraulic flushing as a mechanism affecting the biology and ecology of entire systems has long been recognized (Ketchum, 1951, 1954), and as a bloom-terminating mechanism has been observed for other HABs, particularly blooms of cyanobacteria (Jacoby et al., 2000; Moustaka-Gouni et al., 2006).

In addition to the direct loss of cells through flushing, *P. parvum* likely ceased production of toxins with the rapid increase in nutrient concentrations (Roelke et al., 2007; Errera et al., 2008). Our sampling revealed the complete removal of ambient toxicity to fish with this inflow event. This removal of fish toxicity might have resulted from the cessation of toxin production coupled to degradation of the existing toxins, dilution of in-lake toxin concentrations by hydraulic flushing, or it might have resulted because the pH dropped to levels that altered the ionization state of toxins, rendering them harmless (Valenti et al., in press). Regardless of the mechanism, the removal of toxicity would have greatly diminished *P. parvum*'s ability to compete with other phytoplankton or inhibit zooplankton.

Interestingly, the bloom started to decline (based on direct cell counts) before hydraulic flushing affected the lake. It is unclear why. The predicted growth rate of *P. parvum* based on salinity, temperature and light increased from February to March 2007, mostly because of the temperature increase. Inorganic nutrients did not appear limiting to *P. parvum*. Copepod biomass increased (data not shown), suggesting increased grazing, but these biomass increases were only observed in the upper reaches of the lake, in contrast to the system-wide decrease in *P. parvum*. Factors unaccounted for in this study include pathogens of *P. parvum*. Viruses and algicidal bacteria are known to deleteriously affect other HABs (Kodama et al., 2006; Salomon and Imai, 2006). Furthermore, *in situ* experiments from Lake Whitney, positioned down-river from Lake Granbury, showed that viruses impacted population dynamics of *P. parvum* in later stages of bloom development (Schwierzke et al., in press). While we did not sample viruses in this study, it may be that they influenced *P. parvum* population densities just prior to the impact of the first inflow event.

The post-flushing distribution of phytoplankton was patchy, but at much lower densities than prior to the flushing event of April 2007. Although the conditions of temperature, salinity, and light were predicted to be favorable for *P. parvum* growth, the bloom did not re-establish. This was likely due to the subsequent hydraulic flushing events that occurred in the remaining spring months of 2007. Early during the following summer, when inflows were reduced, the bloom still did not re-establish. Environmental conditions were not as stressful as spring conditions, and thus toxins were likely not being produced, leaving *P. parvum* vulnerable to zooplankton grazers, such as the rotifers that dominated the zooplankton community at this time.

Eventually, conditions again became stressful for *P. parvum* growth as summer temperatures rose above its optimum. Yet still there was no indication of toxin production by *P. parvum*, and *P. parvum* did not become abundant. At this time, however, cyanobacteria were present. There is indirect evidence that some cyanobacteria might have an allelopathic effect on *P. parvum* in Texas lakes (Grover et al., in press; Roelke et al., in press). Thus their presence during summer months of 2007 might have prevented *P. parvum* from accumulating biomass.

This study underscores the importance of hydraulic flushing as a mechanism terminating *P. parvum* blooms in lakes of the south-central USA. In the decades to come, it is likely that the magnitude of lake-flushing events will decrease as human populations expand and the number of water impoundments increases. The effects of decreased through-flow might be exacerbated by climate change as well, as precipitation patterns alter. It may be that the effects of reduced through-flows and likely persistence of blooms can be offset by management efforts aimed at altering other factors influential to *P. parvum* bloom dynamics. For example, blooms might be mitigated by localized fertilization in areas where blooms develop in an attempt to prevent toxin production (Barkoh et al., 2003; Grover et al., 2007; Roelke et al., 2007; Errera et al., 2008), promoting growth of phytoplankton that can suppress *P. parvum* through allelopathy (Grover et al., in press; Roelke et al., in press), manipulations of pH to negate the potency of toxins (Valenti et al., in press), or introduction of natural predators and pathogens (Schwierzke et al., in press). There is much understanding still to be gained, however, before effective management of *P. parvum* in lakes of the south-central USA can be implemented.

Acknowledgements

We thank Mieke Lahousse and Fabiola Urena-Boeck for assistance with laboratory assays. We also thank David Hambright and two anonymous reviewers for comments on a previous iteration of this paper. This research was supported by the Texas Parks and Wildlife Department and by Congressional funding through the U.S. Department of Energy.[SS]

References

Amsinck, S.L., Jeppesen, E., Landkildehus, F., 2005. Inference of past changes in zooplankton community structure and planktivorous fish abundance from sedimentary subfossils—a study of a coastal lake subjected to major fish kill incidents during the past century. Arch. Hydrobiol. 162, 363–382.

Armstrong, F.A., Sterns, C.R., 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Res. I 14, 381–389.

Baker, J.W., Grover, J.P., Brooks, B.W., Ureña-Boeck, F., Roelke, D.L., Errera, R.M., Kiesling, R., 2007. Growth and toxicity of *Prymnesium parvum* (Haptophyta) as a function of salinity, light and temperature. J. Phycol. 43, 219–227.

Baker, J.W., Grover, J.P., Ramachandrannair, R., Black, C., Valenti Jr., T.W., Brooks, B.W., Roelke, D.L., 2009. Growth at the edge of the niche: an experimental study of the harmful alga *Prymnesium parvum*. Limnol. Oceanogr. 54, 1679–1687.

Barkoh, A., Smith, D.G., Schlechte, J.W., 2003. An effective minimum concentration of un-ionized ammonia nitrogen for controlling *Prymnesium parvum*. N. Am. J. Aquacult. 65, 220–225.

Barreiro, A., Guisande, C., Maneiro, I., Lien, T.P., Legrand, C., Tamminen, T., Lehtinen, S., Uronen, P., Granéli, E., 2005. Relative importance of the different negative effects of the toxic haptophyte *Prymnesium parvum* on *Rhodomonas salina* and *Brachionus plicatilis*. Aquat. Microb. Ecol. 38, 259–267.

Brooks, B.W., James, S.V., Valenti Jr., T.W., Urena-Boeck, F., Serrano, C., Berninger, J.P., Schwierzke, L., Mydlarz, L.D., Grover, J.P., Roelke, D.L., in press. Comparative toxicity of *Prymnesium parvum* in inland waters. J. Am. Water Res. Assoc. doi:10.1111/j.1752-1688.2009.00390.x.

Burkholder, J.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8, 77–93.

Calliari, D., Tiselius, P., 2005. Feeding and reproduction in a small calanoid copepod: *Acartia clausi* can compensate quality with quantity. Mar. Ecol. Prog. Ser. 298, 241–250.

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

Chang, F.H., Uddstrom, M.J., Pinkerton, M.H., Richardson, K.A., 2008. Characterizing the 2002 toxic *Karenia concordia* (Dinophyceae) outbreak and its development using satellite imagery on the north-eastern coast of New Zealand. Harmful Algae 7, 532–544.

Edvardsen, B., Paasche, E., 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. In: Anderson, D.M., Cembella, A.D., Hallegraff, G.M. (Eds.), The Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, pp. 193–208.

Errera, R.M., Roelke, D.L., Kiesling, R., Brooks, B.W., Grover, J.P., Schwierzke, L., Ureña-Boeck, F., Baker, J.W., Pinckney, J.L., 2008. The effect of imbalanced nutrients and immigration on *Prymnesium parvum* community dominance and toxicity: results from in-lake microcosm experiments, Texas, US. Aquat. Microb. Ecol. 52, 33–44.

Fistarol, G.O., Legrand, C., Granéli, E., 2003. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar. Ecol. Prog. Ser. 255, 115–125.

Fistarol, G.O., Legrand, C., Granéli, E., 2005. Allelopathic effect on a nutrient-limited phytoplankton species. Aquat. Microb. Ecol. 41, 153–161.

Gordon, N., Colorni, A., 2008. *Prymnesium parvum*, an ichthyotoxic alga in an ornamental fish farm in southern Israel. Israeli J. Aquacult. 60, 5–8.

Granéli, E., Johansson, N., 2003. Effects of the toxic haptophyte *Prymnesium parvum* on the survival and feeding of a ciliate: the influence of different nutrient conditions. Mar. Ecol. Prog. Ser. 254, 49–56.

Granéli, E., Hansen, P.J., 2006. Allelopathy in harmful algae: a mechanism to compete for resources? In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer, Berlin/Heidelberg, pp. 189–202.

Granéli, E., Weberg, M., Salomon, P.S., 2008. Harmful algal blooms of allelopathic microalgal species: the role of eutrophication. Harmful Algae 8, 94–102.

Grover, J.P., 1989. Phosphorus-dependent growth kinetics of 11 species of freshwater algae. Limnol. Oceanogr. 34, 341–348.

Grover, J.P., Sterner, R.W., Robinson, J.L., 1999. Algal growth in warm-temperate reservoirs: nutrient-dependent kinetics of individual taxa and seasonal patterns of dominance. Arch. Hydrobiol. 145, 1–23.

Grover, J.P., Baker, J.W., Ureña-Boeck, F., Brooks, B.W., Errera, R., Roelke, D.L., Kiesling, R.L., 2007. Laboratory tests of ammonium and barley straw extract as agents to suppress abundance of the harmful alga *Prymnesium parvum* and its toxicity to fish. Water Res. 41, 2503–2512.

Grover J.P., Baker, J.W., Roelke, D.L., Brooks, B.W., in press. Current status of mathematical models for population dynamics of *Prymnesium parvum* in a Texas reservoir. J. Am. Water Res. Assoc. doi:10.1111/j.1752-1688.2009.00393.x.

Guo, M., Harrison, P.J., Taylor, F.J.R., 1996. Fish kills related to *Prymnesium parvum* N. Carter (Haptophyta) in the Peoples Republic of China. J. Appl. Phycol. 8, 111–117.

Harwood, J.E., Kuhn, A.L., 1970. A colorimetric method for ammonia in natural waters. Water Res. 4, 805–811.

Holdway, P.A., Watson, R.A., Moss, B., 1978. Aspects of the ecology of *Prymnesium parvum* (Haptophyta) and water chemistry in Norfolk Broads, England. Freshwater Biol. 8, 295–311.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J., Crayton, M., 2000. Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. Can. J. Fish. Aquat. Sci. 57, 231–240.

Kaartvedt, S., Johnsen, T.M., Aksnes, D.L., Lie, U., 1991. Occurrence of the toxic phytoplagellate *Prymnesium parvum* and associated fish mortality in a Norwegian fjord system. Can. J. Fish. Aquat. Sci. 48, 2316–2323.

Ketchum, B.H., 1951. The flushing of tidal estuaries. Sewage Ind. Waste. 23, 198–209.

Ketchum, B.H., 1954. The relation between circulation and planktonic populations in estuaries. Ecology 35, 191–200.

Kirk, J.T.O., 1994. Light and Photosynthesis in Aquatic Ecosystems, 2nd ed. Cambridge University Press, Cambridge, UK.

Kodama, M., Doucette, G.J., Green, D.H., 2006. Relationships between bacteria and harmful algae. In: Graneli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer-Verlag, Berlin, pp. 243–258.

Krasnotshchek, G.P., Abramowitsch, L.S., 1971. Mass development of *Prymnesium parvum* Cart. in fish breeding ponds. Hydrobiologia 7, 54–55.

Kurten, G.L., Barkoh, A., Fries, L.T., Begley, D.C., 2007. Combined nitrogen and phosphorus fertilization for controlling the toxigenic alga *Prymnesium parvum*. N. Am. J. Aquacult. 69, 214–222.

Leibold, M.A., Miller, T.E., 2004. From metapopulations to metacommunities. In: Hanski, I., Gaggiotti, O.E. (Eds.), Ecology, Genetics, and Evolution of Metapopulations. Elsevier, Boston, pp. 133–150.

Lindehoff, E., Granéli, E., Granéli, W., 2009. Effect of tertiary sewage effluent additions on *Prymnesium parvum* cell toxicity and stable isotope ratios. Harmful Algae 8, 247–253.

Lundholm, N., Moestrup, O., 2006. The biogeography of harmful algae. In: Graneli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer-Verlag, Berlin, pp. 23–35.

Mackey, M., Mackey, D., Higgins, H., Wright, S., 1997. CHEMTAX—a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 144, 265–283.

Madden, C.J., Day Jr., J.W., 1992. An instrument system for high speed mapping of chlorophyll-*a* and physico-chemical variables in surface waters. Estuaries 15, 421–427.

McCune, B., Mefford, M.J., 1999. PC-ORD, Multivariate Analysis of Ecological Data. Version 5.0, MjM Software Design.

McCune, B., Grace, J.B., 2002. Analysis of Ecological Communities, 1st ed. MjM Software Design.

Michaloudi, E., Moustaka-Gouni, M., Gkelis, S., Pantelidakis, K., 2009. Plankton community structure during an ecosystem disruptive algal bloom of *Prymnesium parvum*. J. Plankton Res. 31, 301–309.

Moustaka-Gouni, M., Vardaka, E., Michaloudi, E., Kormas, K.A., Tryfon, E., Mihalatou, H., Gkelis, S., Lanaras, T., 2006. Plankton food web structure in a eutrophic polymictic lake with a history of toxic cyanobacterial blooms. Limnol. Oceanogr. 51, 715–727.

Nygaard, K., Tobiesen, A., 1993. Bacterivory in algae—a survival strategy during nutrient limitation. Limnol. Oceanogr. 39, 273–279.

Oh, S.J., Matsuyama, Y., Nagai, S., Itakura, S., Yoon, Y.H., Yang, H.S., 2009. Comparative study on the PSP component and toxicity produced by *Alexandrium tamiyavanichii* (Dinophyceae) strains occurring in Japanese coastal water. Harmful Algae 8, 362–368.

Paerl, H.W., 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. 33, 823–847.

Pinckney, J.L., Paerl, H.W., Harrington, M.B., Howe, K.E., 1998. Annual cycles of phytoplankton community structure and bloom dynamics in the Neuse River Estuary, North Carolina. Mar. Biol. 131, 371–382.

Prakash, S., Lawton, L.A., Edwards, C., 2009. Stability of toxigenic *Microcystis* blooms. Harmful Algae 8, 377–384.

Reynolds, C.S., 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge, p. 535.

Rijn van, J., Shilo, M., 1989. Environmental factors in fish culture systems. In: Shilo, M., Sarig, S. (Eds.), Fish Culture in Warm Water Systems: Problems and Trends. CRC Press, Inc., Boca Raton, FL, pp. 163–177.

Roelfsema, C.M., Phinn, S.R., Dennison, W.C., Dekker, A.G., Brando, V.E., 2006. Monitoring toxic cyanobacteria *Lyngbya majuscula* (Gomont) in Moreton Bay, Australia by integrating satellite image data and field mapping. Harmful Algae 5, 45–56.

Roelke, D.L., Buyukates, Y., 2001. The diversity of harmful algal bloom-triggering mechanisms and the complexity of bloom initiation. Hum. Ecol. Risk Assess. 7, 1347–1362.

Roelke, D.L., Eldridge, P.M., 2008. Mixing of supersaturated assemblages and the precipitous loss of species. Am. Nat. 171, 162–175.

Roelke, D.L., Eldridge, P.M., in press. Losers in the 'Rock-Paper-Scissors' game: the role of non-hierarchical competition and chaos as biodiversity sustaining agents in aquatic systems. Ecol. Model. doi:10.1016/j.ecolmodel.2009.02.005.

Roelke, D.L., Augustine, S., Buyukates, Y., 2003. Fundamental predictability in multispecies competition: the influence of large disturbance. Am. Nat. 162, 615–623.

Roelke, D.L., Errera, R., Kiesling, R., Brooks, B.W., Grover, J.P., Schwierzke, L., Ureña-Boeck, F., Baker, J., Pinckney, J.L., 2007. Effects of nutrient enrichment on *Prymnesium parvum* population dynamics and toxicity: results from field experiments, Lake Possum Kingdom, US. Aquat. Microb. Ecol. 46, 125–140.

Roelke, D.L., Schwierzke, L., Brooks, B.W., Grover, J.P., Errera, R.M., Valenti Jr., T.W., Pinckney, J.L., in press. Factors influencing *Prymnesium parvum* population dynamics during bloom initiation: Results from in-lake mesocosm experiments. J. Am. Water Res. Assoc.

Salomon, P.S., Imai, I., 2006. Pathogens of harmful microalgae. In: Graneli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer-Verlag, Berlin, pp. 271–282.

Schwierzke, L., Roelke, D.L., Brooks, B.W., Grover, J.P., Valenti Jr., T.W., Lahousse, M., Miller, C.J., Pinckney, J.L., in press. *Prymnesium parvum* population dynamics during bloom development: a role assessment of grazers and virus. J. Am. Water Res. Assoc. doi:10.1111/j.1752-1688.2009.00391.x.

Skovgaard, A., Hansen, P.J., 2003. Food uptake in the harmful alga *Prymnesium parvum* mediated by excreted toxins. Limnol. Oceanogr. 48, 1161–1166.

Sopanen, S., Koski, M., Kuuppo, P., Uronen, P., Legrand, C., Tamminen, T., 2006. Toxic haptophyte *Prymnesium parvum* affects grazing, survival, egestion and egg production of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa*. Mar. Ecol. Prog. Ser. 327, 223–232.

Sopanen, S., Koski, M., Uronen, P., Kuuppo, P., Lehtinen, S., Legrand, C., Tamminen, T., 2008. *Prymnesium parvum* exotoxins affect the grazing and viability of the calanoid copepod *Eurytemora affinis*. Mar. Ecol. Prog. Ser. 361, 191–202.

Suikkanen, S., Fistarol, G.O., Granéli, E., 2004. Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermannii* on algal monocultures. J. Exp. Mar. Biol. Ecol. 308, 85–101.

Sunda, W.G., Graneli, E., Gobler, C.J., 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. J. Phycol. 42, 963–974.

TPWD, 2003. *Prymnesium parvum* Workshop Report. Texas Parks & Wildlife Department, Austin, TX. http://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/.

Tillmann, U., 2003. Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquat. Microbial. Ecol. 32, 73–84.

Tillmann, U., John, U., Cembella, A., 2007. On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. J. Plankton Res. 29, 527–543.

Turner, J.T., 2006. Harmful algae interactions with marine planktonic grazers. In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer, Berlin/Heidelberg, pp. 259–270.

Uronen, P., Lehtinen, S., Legrand, C., Kuuppo, P., Tamminen, T., 2005. Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. Mar. Ecol. Prog. Ser. 299, 137–148.

U.S. Environmental Protection Agency, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA-821-R-02-012. United States Environmental Protection Agency, Washington, DC.

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003

Utermöhl, H., 1958. Zur Vervollkominnung der quantitativen phytoplankton methodik. Mitt. Int. Ver. Theoret. Ang. Limnol. 9, 1–38.
Valenti, T.W., James, S.V., Lahousse, M., Schug, K.A., Grover, J.P., Roelke, D.L., Brooks, B.W., in press. A mechanistic explanation for pH-dependent ambient aquatic toxicity of *Prymnesium parvum* Carter. Toxicon. doi:10.1016/j.toxicon.2009.09.014.
Wetzel, R.G., 2001. Limnology, 3rd ed. Academic Press, San Diego, CA, US.
Wright, S., Thomas, D., Marchant, H., Higgins, H., Mackey, M., Mackey, D., 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorization program. Mar. Ecol. Prog. Ser. 144, 285–298.

Please cite this article in press as: Roelke, D.L., et al., Hydraulic flushing as a *Prymnesium parvum* bloom-terminating mechanism in a subtropical lake. Harmful Algae (2010), doi:10.1016/j.hal.2009.12.003